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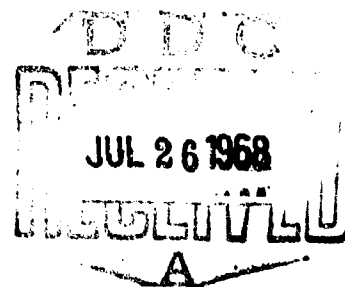
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FAVORABLE INFLUENCE OF THE INFLUENZAL VIRUS
ON ASSOCIATED INFECTIONS

Paul Bordet and L. Quersin-Thiry

Favorable Influence of Influenza Virus
on Associated Infections

Paul Bordet and Lise Quersin-Thiry, Annales de l'Institut Pasteur, Oct. 1951, vol. 81. Translated by Bernice B. MacDonald.

Upon discovering the influenzal virus and recognizing the receptivity of the ferret in this respect, Smith, Andrewes and Laidlaw in 1933 opened the way for the experimental studies of this disease. The following year, the same authors showed that the mouse is equally receptive, and one said that, at least after several transistions, the influenzal virus, inoculated intranasally developed in these animals a fatal pneumonia, consecutive to an intense multiplication of the live virus.

Even if one introduces other wasy, the influenzal virus, upon using a strong dose is capable of killing the mice, or in this case does not multiply in vivo and its effects described in 1946 by G. Henle and W. henle (1) can therefore be considered of a toxic nature. In particular, according to these authors the intracerebral injection of virulent and not diluted allantoic liquid causes toxic and clonic convulsions and carries with it the death of the animal 24 to 72 hours later. Injected by the intra-abdominal or intravenous way this liquid kills the mice in 8 to 96 hours later, on regularly determining the necrotic lesions diffused through the liver and spleen one observes sometimes, in additon, the clogging up of the mesenteric circulation and pulmonary congestion, one notes finally especially the

the mice that survive 2 or 3 years, the presence of pleural effusion, the tentative ways of inoculation of suspensions of injured organs have failed. The toxic effects have specifically hindered the homologous anti-serum, and the preliminary vaccination of mice protect them against it.

In the same article, Henle and Henle point out, without comment, that they have been able to provoke the death of the white rat, guinea pigs and rabbits.

Finally according to these authors the toxicity thus put in evidence seems intimately related to the virus itself; they are not separable neither by centrifugation nor by adsorption on hematites after elution. However, the infectious power of the virus appeared to be more sensitive than the toxic power, to heat, formaldehyde and the UV, without which it would be nevertheless, possible to obtain by the use of these agents, a preparation again toxic with the result that they have lost all infectious power. Another different characteristic between the infectious power and the toxicity consists in that, in the culture in a chicken embryo the first often attains its maximum 24 hours after inoculation whereas the toxicity is not discernable at that moment and is not well marked even after 48 hours.

After the initial work of Henle and Henle, the toxic properties of the influenzal virus have again been made the object of many publications.

In 1948, S. Harris and M. Henle (2) have recognized that

the intravenous injection of virulent allantois liquid causes in the rabbit after 3 hours a marked diminution, exceeding 70 per 100, the number of blood lymphocytes, this lymphocytopenia lasts only a few hours and is not accompanied by a diminution of polynucleus where the phagocytic aptitudes estimated with regard to the dead pneumonocci R, appear to be equally unchanged. This lymphopenia is accompanied by fever. (Wagner, Bennett and Lequire (3)), which after these authors, were caused by a factor probably identical to hamagglutinine.

The intravenous injection of the influenzal virus at this rate, causes after a few minutes a drop in blood pressure (Kempf and Chang (4)), due to a neutralizable factor in the anti-influenza serum and a relatively fixed temperature: it is not destroyed by heating at 56° even after 6 hours.

Henle and Henle have already made known the usefulness, of obtaining very toxic cultures, of inoculating the egg by means of virulent suspension highly diluted. With the same idea McKee (5) showed later that the inoculation in series of non-diluted viruses bring about the obtaining of cultures which although they show a high hemagglutinat titer, do not cause accidents by intracerebral injection of the mice, from the antigenic point of view, the non-toxic viruses seem equivalent to the normal viruses.

The degree of toxicity of the influenzal virus (strain PR8) has been recently defined by Kempf and Harkness (6) with

respect to the ferret. The dose of virus capable of killing the animal by intoxication which one injects intravenously, being correlative to the hemagglutinat titer of the virulent suspension used, can be estimated in hemagglutinat units per gram of animal; the number of units is calculated by dividing by the weight of the animal expressed in grams, the titer of the suspension by the number of cubic centimeters injected. Thus expressed, the dose capable of 75 p. 100 animals in a period of 8 hours- the brevity of this period makes it possible to attribute the death to only the toxic action of the virus-is raised to 154 units. The mice seem very sensitive to intoxication, the fatal dose thus calculated is no more than 53 hemagglutinat units per gram for this animal.

The importance of the role effected by these secondary infections in the importance of influenza in man, confers a particular interest to the experimental study of the problems of sensitization, of the influenzal virus, to the associated bacterial infections. Some of the observations on this subject have been compiled up to the present and they exclusively concern the influenza infection in mice. Thus it is that Harford, Leidler and Hara have stated that, after inoculation of the influenzal virus by respiratory means, the inhalation of pneumococci cause a very serious infection in the mice which in the absence of the virus, in so far as the infection is concerned, has already caused microscopically discernable lesions from the moment the

pneumococci are introduced. On the other hand Carlisle (8) has recognized that the intranasal inoculation of the rat, of an infra-fatal dose of the influenzal virus A lowers the resistance of the animal to the hemolytic streptococci of group C, subsequently introduced the same way, that it is 4 to 12 days after the virulent inoculation that the sensitivity is best marked.

Let us point out finally that Dr. J. Merchand and H.R. Morgan (9) have studied recently the effects of the influenzal virus, in vitro, on the phagocytosis of dead bacteria. It applies to, with the object of, the leucocytes of the guinea pig which, like Mungster, Gordon and Collins (10) have shown dividing with the hematites of this animal the properties of being agglutinable for the influenzal virus. In this mixture of leucocytic suspension and the virulent suspension, they introduced after 15 minutes a suspension of anthrax bacillus killed by heating. After approximately 15 minutes of heating at 37° by determining the percentage of the cells which have surrounded the bacteria, the phagocytosis appears, with respect to mixtures, proves they do not contain virus, lessened in moderate proportion but still appreciable, in a proportion of 23 to 30 per 100. This inhibiting effect of the virus is suppressed when this has been previously neutralized by the homologous anti-serum or when it is heated for 40 minutes at 50°; although this heat respects the hemagglutinating power.

We have begun the study of sensitization by the influenzal

virus in secondary bacterial infections, choosing the guinea pig as the experimental animal, and choosing the intraperitoneal means of inoculation. If it turns out that this animal is not receptive to the influenza infection, such a sensitization would be very striking, and the absence of multiplication of the virus in vivo would permit the attributing of a direct toxic effect of the virus, where the action without doubt could be, in this way, analyzed easily; in addition, the intraperitoneal way is especially propitious for the study of consecutive reactions to the inoculation. In order to determine the secondary bacterial infection, it was indicated to apply a known species as particularly apt to develop in the course of the influenza in man: we have chosen the Pfeiffer bacillus, where the frequency in the course of the influenza is such that it was considered at the time of its discovery as the agent itself of this disease and which has another advantage of not being endowed, with respect to the guinea pig, with a mediocre virulence where the final exhalation would be correlatively easy to detect.

The virus which we are using is influenzal virus A, strain PR8, which was obligingly furnished by Prof. Lepine of the Pasteur Institute. The embryonic eggs, 10 to 11 hours old, are inoculated by the allantois method with the help of 0.1 cm^3 of a 10 to 5 dilution of the virulent allantois liquid. After standing 48 hours at 36° , the embryos are refrigerated for several hours at 4° , and the allantois liquids collected. After a rapid test of hemagglutination, the positive liquids are gathered.

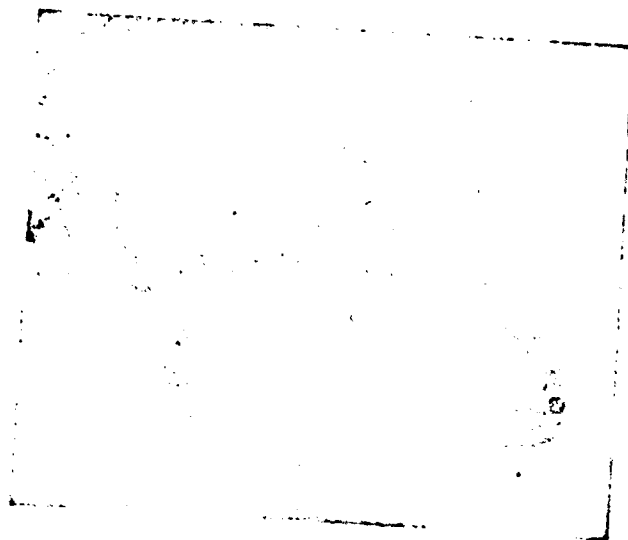
One controls by inoculation the bacteriologic sterility of the mixture thus obtained and one determine its hemagglutinant titer, in regard to the human red blood cells of Group O, by the classic technique recommended by the "Committee on standard serological procedures in influenza studies" (J. Immunol., 1950, 65, 347). According to the portion this titer is 1.512 or 1/1204. The allantois liquid collected in 9 embryos serves as proof.

The strain of Pfeiffer bacillus which we have used, isolated from expectorate proceeding from a case of tracheid, killed the new guinea pig weighing 300 to 325 gr. by intraperitoneal injection, of a dose comprising between 1 and 2 cm³ of culture in blood-bouillon aged 24 hours. In the autopsy, one recovered the bacillus in the peritoneal exudate where it multiplies, and in the heart blood, in which one can place in evidence by inoculation on blood agar.

One easily demonstrates, by the experiments of the type which we have described below that the previous inoculation of the guinea pig with the influenzal virus makes it sensitive to the infection by the Pfeiffer bacillus strain.

Five guinea pigs are used, weighing between 300 and 325 gr. Five cm³ of virulent allantois liquid is injected intraperitoneally to 3 of them and the other 2 the same volume of normal allantois liquid. After 15 to 30 minutes one guinea pig from each group receives the same way 0.5 cm³ and the other 0.75 cm³ of the culture of Pfeiffer bacillus in blood-bouillon aged 24 hours. The 2 guinea pigs inoculated with influenzal virus

GRAPHIC NOT REPRODUCIBLE



Microphotograph of the peritoneal exudate retained 24 hours after injection of 40,960 hemagglutinant units of influenzal virus in the guinea pig: one notices the high proportion of polynuclear to pycnotic nuclei. Dye: blue of toluidine.

after the Pfeiffer bacillus, die: those which received 0.75 cm³ of the culture after 17 hours and those which received 0.5 cm³ after 48 hours. Both show in the autopsy an abundant and fluid peritoneal exudate, in which the Pfeiffer bacillus multiplied; the pleural cavity contains an abundant serous exudate where the inoculation of media developed a culture of Pfeiffer bacillus; the culture of heart blood is equally positive. The guinea pigs that received only the influenzal virus survived. They are the same ones injected with normal allantois liquid after the Pfeiffer bacillus. A puncture of the preitoneal cavity, done on these animals 24 hours after inoculation, yields very little exudate while the limited quantity and the thick consistency contrast to the abundance and fluidity of the exudate which appeared in the animals inoculated with influenzal virus, in addition, the exudate collected in the test animals doesn't show Pfeiffer bacillus, in the others the bacteriologic recovery is then realized on the next day after inoculation.

The production of an abundant and fluid peritoneal exudate results from the action of the influenzal virus, one also observes in the animals inoculated with only virus that in them, in addition, Pfeiffer bacillus are recovered. On the other hand, the same after injection of normal allantois liquid, the peritoneal cavity does not contain, the next day, but a small quantity of thick exudate, rich in leucocytes of normal appearance. Of the first experiments we have noted, on the contrary, the abnormal frequency, in the exudates collected in the animals

inoculated with influenzal virus, of leucocytes with pyknotic nuclei. This peculiarity is clearly marked when recovered as virulent material and eluted virus.

After having carried out many tests on a form analogous to that which has been described, we are in effect exclusively recommending for our subsequent experiments, virus extracts of allantois liquid culture by adsorption on the hemagglutinins following elution: the procedure offers the double advantage of eliminating the proper constituents from allantois liquid and permitting the inoculation of the virus in very large quantities. The preparation of eluted virus has been effected as follows:

The human red blood cells, obtained by centrifugation of blood citrate of Group O are washed three times in physiologically sterile solution afterwards the addition of virulent allantois liquid recently collected, in such quantity that it is brought to a concentration of 2 p. 100. After a period of 2 hours at 4° in the course of which repeatedly it is agitated lightly in order to put the agglutinated cells in suspension and thus facilitate the adsorption of the virus, the mixture is subjected to a short centrifugation at 4,000 rpm. The residue of cells is collected in a volume of physiological solution 9 to 10 times smaller than the initial virulent liquid, and the elution is done by putting the globular suspension in a water bath at 36° where it is maintained for 2 hours. One removes the corpuscles by a short centrifugation and decanting of the supernatant liquid which contained the eluted virus: this liquid is lightly opa-

lescent and toned with hemaglobin. Before using, the bacteriological sterility is controlled and one titers the hemagglutinant power according to the classic technique: according to the amount the titer is found to be between $1/8192$ and $1/32768$. A test liquid is obtained by centrifugation, following decanting, of a suspension of corpuscles having remained, likewise, in a normal allantois liquid.

A strong dose of this eluted virus is capable of killing the guinea pig by intoxication, and the inoculation of an infra-fatal dose permits the Pfeiffer bacillus introduced again of causing, besides the dose of $0,25 \text{ cm}^3$ of culture, an infection often fatal, in less than 24 hours. While underlining from the present that the favorable influence of the virus in regard to the bacterial infection appears as a consequence of its toxic action, we show from the first the relative data on the toxicity of the virus inoculated alone, and will only consider again those which concern the increase of sensitivity to the bacterial infections.

Of course the toxic effects of the virulent liquid are in general much more marked when the liquid is injected in very large quantities and whose titer is very high. Also it is convenient, in order to define the dose of virus inoculated, to express it in hemagglutinat units, where the name corresponds to the product of the hemagglutinat titer of the suspension by the number of cubic centimeters injected.

The guinea pigs used, whose weight always is between 300

and 325 gr., have regularly survived the intraperitoneal injection of a virulent liquid of a titer equal to $1/8129$ or therefore an inoculation of 20,840 hemagglutinat units. But the injection of this dose, we observed it, is quadruple from the point of view of hemagglutinat units of that placed in the eggs in the experiments recorded very high and allowed the injection of 5 cm^3 of virulent allantois liquid whose titer does not exceed $1/1024$ suffices to make disappear, in a constant and very marked manner, the effects most characteristic of the action of the virus.

The most apparent effect of this inoculation already ascertained during the use of the non-eluted virus, consisting in the production of a peritoneal exudate, very abundant and fluid, coming out under pressure when the peritoneal cavity of the animal is punctured 24 hours after inoculation, while the inoculation of a test liquid, which does not allow, after this delay, only a small amount of thick exudate. Upon microscopic examination the latter shows numerous normal looking leucocytes. One is affected on the contrary upon microscopic examination of the exudate collected from the guinea pigs inoculated with virus, by the frequency, very clear on the microphotograph joined to our text, of pycnosis images which show the leucocytes. Frequently the nucleus appears extremely divided, in corpuscles which the number can exceed 10. These pycnosis leucocytes are not manifested in the guinea pig inoculated with virus after 24 hours or longer. If, as it occurs often in animals having received other viruses,

the Pfeiffer bacillus, death follows after a short delay, about 18 hours later for example, the appearance of leucocytes is normal; the pycnosis appears to be like the slow monophagæc proof of the alteration stamped on the leucocyte by the virus. After 48 hours, the exudate delay habitually abundant and fluid and appears again to have again numerous leucocytic pycnosis images. Nevertheless, from this moment on the altered leucocytes become the prey of macrophages, whose predominance in the exudate, which becomes less abundant, become more defined in the following days. If the animal has been inoculated with a fatal dose of virus one finds in addition, in the autopsy, a pleural exudate often very abundant and poor in leucocytes, one also notices the hypermia of the suprarenal capsule and the lungs frequently show a fine hemoragic pic.

The dose of virus capable of killing 3 out of 5 guinea pigs by intraperitoneal injection is difficult to fix precisely. Of 8 guinea pigs which received a dose of virus corresponding to 40,960 hemagglutinat units, only one died: death came after 48 hours; the peritoneal and pleural exudates, as well as the heart blood, appeared sterile by inoculation on blood-gelose. It appeared necessary to us, to cause death in the majority of the cases, by inoculating a quadruple dose of the former or 163,840 units, that is to say, approximately 550 units per gram of animal weight. We recall in this connection that Kempf and Harkness, while using the intravenous method, have estimated the dose determining death by intoxication and expressed it in

hemagglutinat units per gram at 154 units for the ferret and 53 units for the mice.

We have followed, in certain guinea pigs, the lot in vivo inoculated at the same time determining the hemagglutinat activity of the exudate and in infecting the latter in embryos. For this purpose, the exudate added to a solution of sodium citrate, collected in addition when it must be inoculated, 1,000 units of penicillin and 500 units of streptomycin per cm^3 . It is inoculated with a dose of 0.1 cm^3 , either as it is or partially diluted to respective proportions of 10-2, 10-4, 10-6. each dose being injected in 2 eggs. When the allantois liquid, collected 2 days after inoculation, seems hemagglutinat, one ascertains by the vitality of the virus.

In a guinea pig having died 22 hours after the inoculation of 163,840 units, the peritoneal exudate collected in the autopsy had a hemagglutinat titer of $1/2048$, for the pleural exudate the titer was $1/8$. In these exudates as well as in the heart blood, the virus could in addition be used as culture. But the persistence of the virus in vivo could exceed 24 hours. This is what the results provided by 2 other guinea pigs show, which having received the same dose (163,840 units) were still alive 48 hours after inoculation; of the two guinea pigs 1 (A) dies 52 hours later, the other (B) recovers. The hemagglutinat titer of the peritoneal exudate collected 48 hours later is for A, $1/1024$ and for B, $1/512$. The inoculation of the exudate in the eggs, the same diluted 10-6, furnished all positive cultures. In one final

case, the inoculation of heart blood drawn after 3 days development in 2; as to the liquids collected before the third day, our results have been negative. We point out also that the use of virulent allantoic liquid proceeding from embryos inoculated with exudate does not make evident any exhalation of the toxicity, for the guinea pigs, of virus having passed through this animal. Finally, the intraperitoneal injection of 20,480 units of influenzal virus B (Lee strain) without causing death in the animal. caused, like a dose equivalent to PR8, the appearance of an abundant exudate characterized by the frequency of leucocytic pycnosis images.

As we have pointed out above, the use of eluted virus makes appear very plainly that this one with virulent allantoic liquid increases the sensitivity produced by the virus in regard to the Pfeiffer bacillus.

In effect, when one injects the Pfeiffer bacillus in the peritoneal cavity 15 to 30 minutes after having introduced the eluted influenzal virus, the fatal dose is found to drop to approximately 0.25 cm^3 , after having received an infra-fatal dose of eluted virus (according to the case 20,480 or 40,960 hemagglutinat units) 7 dies, of which 4 in less than 24 hours and 3 in approximately 36 hours. In addition in the 4 guinea pigs which have survived , the inoculation of the peritoneal exudate, drawn 24 hours after inoculation regularly develops a culture of Pfeiffer bacillus, twice on continuous beds and 2 in isolated colonies. On the other hand, the guinea pigs inoculated

with proof liquid, then the culture of Pfeiffer bacillus, in doses of 0.25 or 0.5 or with 1 cm³ dies. In these guineapigs, in addition, the peritoneal exudate, collected 24 hours later and inoculated on blood-gelose, seem sterile when the dose of culture injected was 0.25 or 0.5 cm³. In the case that it was 1 cm³ the exudate collected 24 hours later, contained many rare germs developed by inoculation in not very numerous isolated colonies. The test guinea pigs die, on the contrary, after 2 or 3 days if the dose of culture injected is 1.5 cm³.

These results lend to fixing approximately between 4 and 6 the coefficient of increase, under the action of the influenzal virus, of the sensitivity of the guinea pigs to the Pfeiffer bacillus. This marked variation seems extremely significant, if one takes into account, that the influenzal virus does not multiply in the body of the guinea pig, and one knows that, on the species receptive to the influenza infection the increase of sensitivity remains this way- and it proves itself therefore very clear again- under the action of the virus which reproduces in vivo. In the guinea pig, on the other hand, the action of the virus is not without doubt temporary; i. e. effect, its favorable influence on the infection by the Pfeiffer bacillus does not distinguish that condition that develops separating the injection of virus from that of the bacteria may be quite brief. In the great number of our tests this delay has been of 15 to 30 minutes. We have stated that it can be disposed in 3 hours without the increase of sensitivity ceasing to be evident. On the other

hand, when one injects a dose of 0.25 cm^3 in the peritoneal cavity of a guinea pig inoculated with influenzal virus 24 hours earlier, the Pfeiffer bacillus is destroyed the day after just as it had been injected in a new guinea pig; the inhibition of the virus on the peritoneal defense is then dissipated after 24 hours.

The guinea pigs inoculated with eluted influenzal virus then with Pfeiffer bacillus, show the characteristic signs of the action of the virus alone; they contain an abundant and fluid peritoneal exudate, show (a condition like we have already mentioned, in which death does not follow in less than 24 hours) of very numerous leucocytic pycnosis images, one finds, in addition, an abundant serous pleural exudate poor in leucocytes. In the autopsy the Pfeiffer bacillus multiplies in the peritoneal exudate, and it seems also in less numbers a culture in continuous beds or in confluent colonies; the inoculation of heart blood develops numerous colonies. In the test guinea pigs inoculated with Pfeiffer bacilli, the peritoneal exudate, removed after 24 hours is not abundant, thick and rich in normal looking leucocytes; the animal does not die, we recall it, that if the dose of the culture is 1.5 cm^3 , in this case the infection is equally generalized and the bacilli can be placed in evidence in the heart blood.

In conclusion using such a dose that it is destroyed in less than 24 hours in the test guinea pig, the Pfeiffer bacillus not only remains but very often multiplies abundantly

in the peritoneal cavity of the guinea pig having received little before an infra-fatal dose of influenzal virus; it colonizes the pleural overflow which the virus causes the formation of, the animal dies of septicemia.

Finally we point out that this favorable influence on the associated bacterial infections is attested with the same clarity when the virus inoculated belongs to type B (Lee strain).

Although perceptible in the guinea pig, the favorable influence on the infection by the Pfeiffer bacilli therefore is clearly marked immediately after the inoculation of the virus. Being immediate but only passing, it appears to be tied in with the direct toxic effects of the virus, which, however, does not reproduce in the organism of the guinea pig, where it vanishes quickly. But, the inoculation of virus causes the formation of an abundant serous exudate, whose appearance under the microscope is characterized by the great frequency of leucocytic pycnosis images. Without a doubt it is likely, a priori, that this alteration evident in the polynucleus explains, at least in part, the increase of sensitivity in regard to the associated bacterial infections, and the phagocytosis appeared to us effectively more active, after the injection of the Pfeiffer bacillus, in the test guinea pig than in those previously inoculated with virus. But, a firm conclusion in this connection would not be permissible except after the study which we will undertake shortly, of the action of the virus on the phagocytosis in vitro. Other research now in progress, concerns the influence of the inocu-

lation previous to the virus on the infection by bacteria other than the Pfeiffer bacillus.

We concern ourselves, on the other hand, with specifying the relations which connect the favorable influence of the virus to its virulence and to its hemagglutinat power. The results obtained to the present, indicate that it is annuled by heating the eluted virus for $\frac{1}{2}$ hour at 60° ; it is not affected on the contrary by heating for 40 min, at 50° , which destroys the concerning factor in the action described by Merchant and Morgan and which we have related above. But, our efforts, with the object of separating either the virulence or the hemagglutinat power have collided up to the present with those difficulties recalling those which G. Henle and W. Henle have noted in regard to the toxicity of the influenzal virus on mice.

SUMMARY

In order to refer to the experimental study the favorable influence, well known in human clinic, that the influenza exerts in regard to certain bacterial infections, it has appeared useful to find out from the very first if this influence can be observed the same in a species not receptive to the influenzal virus, in the affirmative, in effect, it can be referred to a direct toxic action of the virus. Also we have had to resort to the guinea pig, in which the influenzal virus does not reproduce. As an agent associated bacterial infections we have used a strain of Pfeiffer bacilli, which one says, that it is often abundant in the course of influenza, to the point that it was

considered at the time of its discovery as the agent itself of this disease. In this work, we described the effects of intraperitoneal injection of the influenzal virus and submit in evidence the increase of sensitivity which this inoculation determines in regard to the Pfeiffer bacilli, introduced the same way.

1) The most apparent effect, very perceptible after the injection of 5 or 10 cm³ of virulent allantois liquid with a hemagglutinat titer equal to 1/512 or 1/1024, consists in the formation of an abundant and fluid peritoneal exudate, which does not appear after the injection of normal allantois liquid. This dose already permits the recognition of the favorable influence of the virus on the infection by the Pfeiffer bacilli.

2) Allowing the injection of large quantities of virus, the use of eluted virus after adsorption on the hemagglutinins, is particularly favorable as evidence of the effects which it exercises. The injection of 20,000 and 40,000 hemagglutinat units, without causing the death of the animal, causes an abundant and fluid peritoneal exudate to appear regularly, coming out under pressure of the peritoneum is punctured 24 hours after inoculation. Upon microscopic examination of this exudate one is impressed by the great frequency of leucocytic pycnosis images. This injection causes, in addition, the appearance of an abundant serous pleural exudate low in leucocytes.

3) The dose of virus capable of killing the majority of the guinea pigs appears to be 160,000 hemagglutinat units:

calculated per gram of animal weight, it could therefore be approximately estimated at 500 units.

The culture has permitted to place in evidence the virus in the exudate and the heart blood 24 to 48 hours after inoculation we have not found it beyond the third day.

4) The guinea pigs having received an infra-fatal dose of influenzal virus (either 20,00 or 40,000 hemagglutinat units) by the peritoneal method, show, in regard to the Pfeiffer bacilli, introduced the same way 15 to 30 minutes later, a sensitivity 4 to 6 times greater than that of the test animals. This increase of sensitivity is marked the same way when the delay between the injection of the virus and that of the bacteria, is carried to 3 hours: it is not observed when the Pfeiffer bacilli is injected 24 hours after the virus.

↓ The high proportion of polynucleus pycnosis in the exudates collected from the guinea pigs inoculated with virus, suggests that the favorable influence of the virus on associated bacterial infections finds its cause at least in part in a diminishing of the phagocytic defenses of the peritoneal cavity, we tried to elucidate this point of phagocytosis in vitro. ↗

This influence is abolished by heating the virulent suspension at 60° for $\frac{1}{2}$ hour, it is not affected by heating for 40 minutes at 50°; the research in progress aspires to determine exactly the relations which unite the virulence and the hemagglutinat power.

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